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Award Number: DAMD17-03-1-0244

TITLE: ERα and ErbB-2 Cross-Talk in Mammary Tumorigenesis and Metastasis

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REPORT DATE: April 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20050824 126

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2005	Annual (31 Mar 2004 - 30 Mar 2005)

4. TITLE AND SUBTITLE
ERα and ErbB-2 Cross-Talk in Mammary Tumorigenesis and Metastasis

5. FUNDING NUMBERS
DAMD17-03-1-0244

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The induction of human breast cancer involves the complex interplay of hormones and growth factor Receptors. The major focus of our DOD sponsored research program is to investigate the interaction of the ERα and erbB-2 receptor in the induction of breast cancer . In addition, we are also interested in the involvement of ErbB-2 coupled signaling pathways such as c-Src and Akt-1 in ERα induced mammary tumors. To accomplish this we have recently derived transgenic mice that expressed high levels of ERα in the mammary epithelium. To explore the significance of erbB-2c-Src and Akt-1 in mammary tumor progression we have recently initiated intercrosses between mice expressing these signaling molecules in the mammary epithelium and the MMTV/ERα strains. The results of these analyses should provide important insight in the mechanism by which erbB-2 and ERα communicate in the induction of breast cancer.

14. SUBJECT TERMS
ERα, ErbB-2, mammary tumorigenesis

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

The major goal of our DOD sponsored research program has been directed towards understanding the importance of crosstalk between the erbB-2 receptor tyrosine kinase and ERα receptors. The basis for these experiments derives from the observation that in addition to well documented role of ERα as a transcriptional activator, ERα has been shown to mediate a number of non-genomic effects that involve physical complexes with the c-Src tyrosine kinase (2). Given that c-erbB-2 is also known to activate c-Src tyrosine kinase (3, 4), it conceivable that c-Src may play a critical role in mediating cross-talk between ERα and ErbB-2. To further test the importance of ERα in ErbB-2 mediated mammary tumorigenesis, one of the major goals of this proposal was investigate whether mammary epithelial specific expression of ERα may modulate mammary tumor progression in the MMTV/ErbB-2 strains. To accomplish these goals, we have recently derived transgenic mice that express an constitutively activated version of ERα under the transcriptional control of the MMTV promoter. To further explore the significance of cross talk of ERα and ErbB-2 in the last renewal period we have crossed the MMTV/Era mice with separate strains of transgenic mice expressing ErbB-2 under the its endogenous or MMTV promoters. In addition, we also assessed whether ErbB-2 coupled signaling molecules such as c-Src and Akt could act synergistically with ERα to accelerate mammary tumor progression. This was accomplished by interbreeding the MMTV/activated ERa mice with separate strains of transgenic mice expressing activated forms of Akt-1 or c-Src under the transcriptional control of the MMTV promoter.

Body

Mammary specific expression of ERα is not associated with mammary tumor induction.

As reported in our first annual report, thee transgenic mice express high levels of ERα in the mammary epithelium. To confirm that these strains expressed high levels of ERα , we performed real time polymerase chain reaction (RT-PCR) on RNA derived from either MMTV/ERα or control FVB mice. The results revealed that MMTV/ERα mice expressed elevated levels of ERα transcript in the mammary epithelium. Consistent with RT-PCR results, immunostain analyses with ERα specific antisera revealed robust nuclear staining (Figure1). Despite the high levels of ERα expression in the mammary

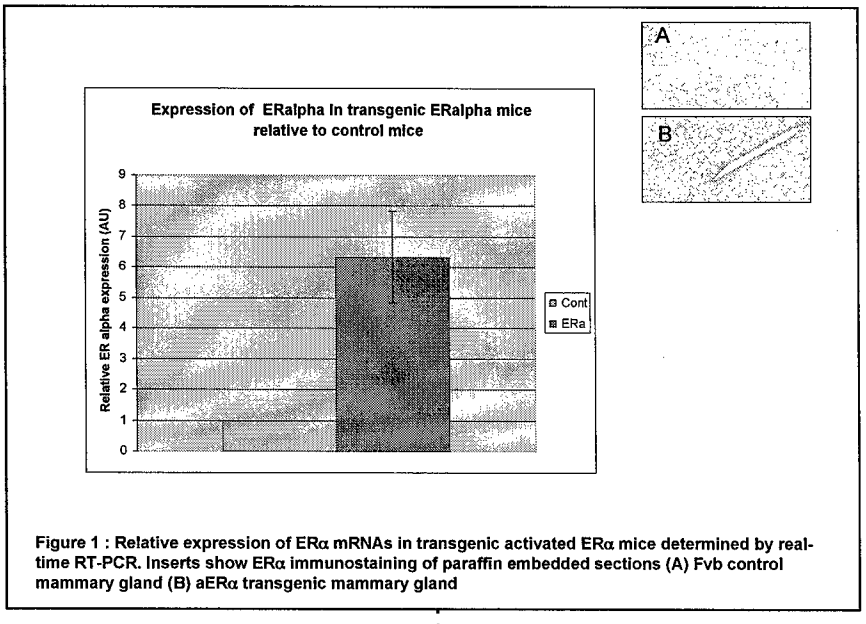


Figure 1 : Relative expression of ERα mRNAs in transgenic activated ERα mice determined by real-time RT-PCR. Inserts show ERα immunostaining of paraffin embedded sections (A) Fvb control mammary gland (B) aERα transgenic mammary gland

epithelium, female mice carrying the MMTV activated ERα have yet to develop mammary tumors.

Generation of bitransgenic animal co-expressing Neu, Akt-1 and c-src with activated ERα in the mammary epithelium

Gentoype	Number of transgenic females	Range of ages
aERα *[1] Strain 02-351-5 Strain 02-346-1 Strain 02-347-2	85 45 20 20	2 to 12 months 2 to 12 months 2 to 12 months
aERα/NeuNDL2-5*[2]	20	2 to 8 months
aERα/FloxNeoNeuNT/MMTV -Cre*[3]	3	2 to 4 months
aERα/Src2*[4]	24	2 to 10 months
aERα/Akt7*[5]	5	2 to 6 months

*1 Activated form of human Estrogen Receptor alpha under the control of the MMTV promoter
*2 Activated ErbB2 under the under the control of the MMTV promoter
*3 Activated ErbB2 under the control of its endogenous promoter
*4 Activated form of Src under the control of the MMTV promoter
*5 Activated form of Akt-1 under the control of the MMTV promoter

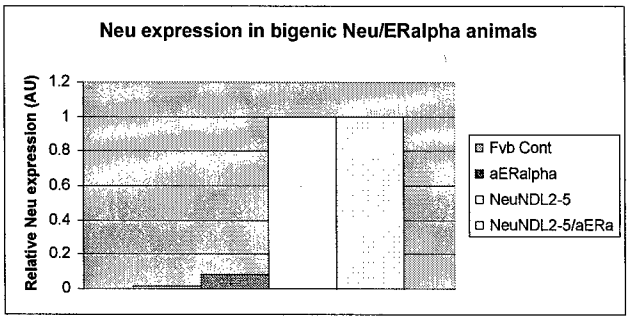
Table 1 : Summary of generated transgenic mice

Genotype	Tumor onset (days)	Total number of animals
NeuNDL2-5	168	3
NeuNDL2-5 / aERα	170	5

Table 2 : Tumor onset in bigenic animals compared to activated Neu transgenic mice (NeuNDL2-5)

To assess whether co-expression of activated ERα and Neu could impact on tumor induction, the MMTV/ERα were interbreed either with separate strains of mice carrying MMTV/activated erbB-2 (Neu NDL2-5, Table1) or mice carrying activated erbB-2 under the endogenous erbB-2 promoter (Flox Neu-NT/MMTV/cre, Table #1). To date we have generated a cohort of 20 virgin females harboring both MMTV/activated erbB-2 and MMTV/ERα mice and 3 females co-expressing ERα in the context of endogenous ErbB-2 promoter (Table #1). In addition to these crosses we have also generated transgenic crosses in which key downstream signaling pathways such s c-Src and Akt-1 are coexpressed in the mammary epithelium with ERα(ERα/c-Src, ERα/Akt7, Table#1). Although we are still in the early stages of analyses for these various crosses, there are

preliminary indication that co-expression of erbB-2 and ERα does not significantly impact on tumor induction in these strains since the bi-genic mice are developing mammary tumors within a similar latency period (Table #2).



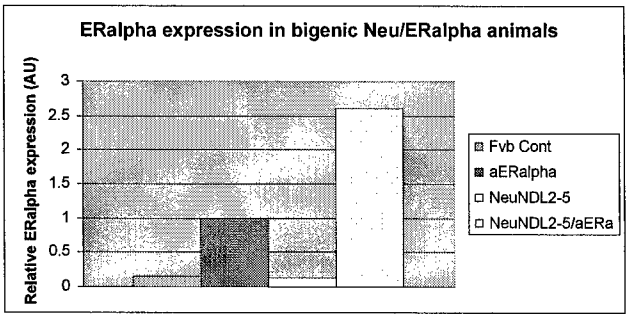


Figure 2 : Expression of Neu and ERα mRNAs in bigenic NeuNDL2-5/aERα mammary glands determined by real-time RT-PCR

The lack of accelerated tumor phenotype was not due to lack of expression of either ERα or Neu as the bigenic mammary glands expressed elevated levels of Neu and ERα transcript compared to the FVB control tissues (Figure 2). Histological analyses of mammary tumors derived from the Neu/ERα revealed that they had comparable pathological phenotypes.

Key Research Accomplishments

1. Generation of bigenic female mice coexpressing ERa and ErbB-2 in the mammary epithelium.
2. Initial characterization of bigenic ERa and erbB-2 tumor phnotype.
3. Generation of bi-genic strains co-expressing activated c-Src and ERa in mammary epithelium
4. Generation of bi-genic strains co-expressing Activated Akt-1 and ERa

Reportable Outcomes

Derivation and characterization of the MMTV/ERa strains.

Conclusions

Although these analyses are based on relatively small cohorts of transgenic animals (Table #2), the future characterization of remaining cohort of animals should provide important insight into the role of ERα in ErbB-2 induced mammary tumor progression. Given the that ERα is known to be involved in the transcriptional regulation of erbB-2 promoter (1), the most informative cross will be female mice expressing ERa in the context of endogenous erbb-2 promoter (Table #1). While we have a cohort of only 3 female, we are in the process of expanding these mice.

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